REMARKS

Specification:

Amendments have been made to the specification to correct minor typographical errors.

Status of the claims:

Claims 9-33 are pending. Claims 15-29 were withdrawn from consideration, subject to applicants' right to pursue the subject matter of these claims in a separate application, and the remaining claims (9-14 and 30-33) stand rejected. No new matter is presented in the foregoing amendments.

Claim construction:

The Examiner objected to the definition and use of the terms "first DNA molecule" and "second DNA molecule" in the claims, as exemplified by Claim 9. The use of these open-ended terms along with language relating to "at least 80% identity" was asserted to be confusing, and encompassing an indeterminable number of sequences, because they were defined using the word "comprising," instead of the term "consisting of" which is not open-ended.

Claims 9 and 10, which have similar language, were amended to clarify their intended scope, substituting the phrase "DNA segment" for the phrase "DNA molecule" and to narrow the scope of the second DNA segment to encompass nucleotide changes primarily in the promoter/enhancer region upstream from a functional reporter gene. Claim 9 (incorporating edits) now reads as follows:

> 9. (Currently amended) A host cell transformed with a reporter nucleic acid comprising a first DNA segment consisting of nucleotides 3005-4336 of SEQ ID NO:1 contiguous to nucleotides 1-243 of SEQ ID NO:1, or a second DNA segment which is at least 80% identical to said first DNA segment across

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nucleotides 3005-3484 of SEQ ID NO:1 and having the same reporter and mRNA terminator function as said first DNA segment.

Claim rejections under 35 U.S.C. § 112, first paragraph:

Claims 9-14 and 31-33 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

The Examiner asserted that the claims embrace almost any modification of the promoter and reporter gene sequences explicitly disclosed in the application, which would encompass a vast genus of undefined sequences. The Examiner indicates the written description requirement for a claimed genus may be met through "a sufficient description of a representative number of species by actual reduction to practice . . . or by disclosure of relevant characteristics . . . sufficient to show that the applicant was in possession of the claimed genus." However, the Examiner asserted that the specification teaches that the nucleic acid comprising nucleotides 3005-4336 of SEQ ID NO:1 has a reporter function which provides for quantitative assessment of β-catenin and Lef-dependent transcription, but since the claim recites "that the nucleic acid having 80% identity with the first DNA molecule 'has the same reporter function as said first DNA molecule,'" then the claim itself does not specify a particular reporter activity for any nucleic acids of the claims, and that a genus representative of such breadth is not described in the application.

If the function is meant to refer to a reporter of β-catenin and Lef-dependent transcription, then the Examiner asserts that the single species disclosed in the application is not sufficiently representative of a broad genus of species as currently claimed in the application. He also stated that applications seeking to claim biomolecules having a defined function, but broadly divergent structure, must disclose a correlation between that function and a corresponding structure, and that no such correlation or relevant identifying characteristics of the claimed invention were found in the application.

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Independent Claims 9 and 11 were amended to narrow their scope, as noted above. Claim 10 depends from Claim 9, and Claims 30-33 directly or indirectly depend from Claim 11. Accordingly, reconsideration and withdrawal of this ground of rejection are respectfully requested.

The Examiner also rejected Claims 9-14 and 31-33 as failing to comply with the enablement requirement. The Examiner stated that although the disclosure is enabling for construction of a specific arrangement of DNA that functions as a reporter of β-catenin and Lef-dependent transcription, host cells harboring the construct, and transgenic zebrafish comprising the host cells, it does not reasonably provide enablement for the broad scope of what is presently claimed.

The Examiner cited several references relating to the level of predictability in the prior art. Pietrzkowski was cited for the teaching that the mutated enhancers reduce promoter activity. Chan was cited for the notion that a mutation in one element of a promoter can abolish activity, even though mutations in other elements of the promoter do not. Omilli was cited as teaching the importance of the relative arrangements of promoter elements as contributing to the activity of the whole promoter. Arnone was cited as teaching that promoters are comprised of a variety of regulatory elements which work in concert to provide the functional characteristics of any given promoter.

Pietrzkowski discloses the characterization of an enhancer-like sequence located between positions -73 and -45, upstream from the transcriptional start site for the human proliferating cell nuclear antigen (PCNA) gene. Synthetic promoters containing mutations in this region lost their ability to drive transcription of heterologous cDNAs. Gel shift and methylation interference experiments demonstrated that the enhancer-like sequence binds nuclear proteins at specific sites in the sequence.

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Chan identified four novel *cis*-acting elements that are involved in the light regulation of the nuclear GAPB gene, which encodes the B subunit of chloroplast glyceraldehyde-3-phosphate dehydrogenase of *Arabidopsis thaliana* using linker scanning and deletion analysis techniques. Mutations in one region (XIII) resulted in a total abolishment of light-activated gene transcription, while mutations in three others (PI, Tboxes, PII boxes) resulted in reductions in promoter activity. Chan concluded that there is a very complex plethora of *cis*-acting elements existing in different light-regulated promoters, even among closely related genes, and that few of these elements are found in all light-regulated genes.

Omilli used quantitative S1 nuclease analysis to study the sequences involved in the initiation of SV40 late gene expression which occur before the onset of viral DNA replication. Deletion or translocation of sequences from a GC-rich domain modulated the level of transcripts initiated at natural late initiation sites, suggesting that the relative arrangement of various genetic elements in a promoter is a critical factor in determining the relative strength of early and late promoters.

Arnone provides a detailed state-of-the art review of *cis*-regulatory systems that controls the expression of genes during development. The genetic elements that comprise these systems in different species may be large or small, may overlap with other genetic elements, may bind proteins which modulate transcriptional activity, or interact directly with other genetic elements over hundreds or thousands of DNA base pairs through DNA looping or other means. The diverse chemical nature and modular structure of these genetic elements almost guarantees that the regulatory controls for any given transcriptional unit are complex, with typical interactions ranging between four to eight different factors.

Independent Claims 9 and 11 were amended to narrow their scope, as noted above. Claim 10 depends from Claim 9, and Claims 30-33 directly or indirectly depend from Claim 11.

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Accordingly, reconsideration and withdrawal of this ground of rejection are respectfully requested.

The Examiner also asserted that the amount of direction provided by the application is inadequate. He indicated that a first DNA molecule comprising a portion of the pTOPFlash vector, containing a TCF/Lef responsive promoter, linked to a dGFP reporter gene (cited above) was disclosed, along with a SV40 polyA signal, and that nucleotides 3005-4336 of SEQ ID NO:1 have a reporter function suitable for assessment of β-catenin and Lef-dependent transcription, but stated that the application includes no teachings as to how the disclosed sequences or genetic elements might be modified while maintaining the same reporter function.

The Examiner also asserted that the structural limitations recited in the claims encompass many thousands of possible combinations and that many, if not most, of these combinations would be inoperative. Since a large amount of effort would be required to determine the operative embodiments within the present scope of the claims, the effort is clearly "undue," and the specification is not enabling for what is presently claimed.

Independent Claims 9 and 11 were amended to narrow their scope, as noted above. Claim 10 depends from Claim 9, and Claims 30-33 directly or indirectly depend from Claim 11. Accordingly, reconsideration and withdrawal of this ground of rejection in view of these amendments are respectfully requested.

Claim rejections under 35 U.S.C. § 112, second paragraph:

Claims 9-14 and 31-33 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that the applicants regard as the invention. The Examiner stated that it is not possible to definitely determine what is in the scope of the nucleic acid of the claims and, therefore, the meets and bounds of the claims as a whole are unclear. An amendment identifying the reference sequence,

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such as "comprising a sequence 80% identical to the nucleotide sequence 3005-4336 of SEQ ID NO:1" would overcome the rejection.

Independent Claims 9 and 11 were amended to narrow their scope, as noted above. Claim 10 depends from Claim 9, and Claims 30-33 directly or indirectly depend from Claim 11. Accordingly, reconsideration and withdrawal of this ground of rejection in view of these amendments are respectfully requested.

Claims 13 and 14 were rejected as being indefinite in reciting "the mutation," where there is no antecedent for a "mutation" in Claim 11, from which the claims depend.

Claims 13 and 14 were amended to depend from Claim 12, which contains the phrase "induced mutation."

Claim rejections under 35 U.S.C. § 102(a):

Claims 9-11, 30, and 31 stand rejected under 35 U.S.C. § 102(a) as being clearly anticipated by Dorsky et al., *Dev. Biol.* 241:229-237 (2002) (published online on February 28, 2002). Dorsky et al. was cited as apparently the applicants' own disclosure of the TOPdGFP vector and transgenic fish (in Figures 1 and 2, and in the materials and methods section beginning on page 230). The Examiner asserted that the cells and transgenic fish of Dorsky et al. clearly anticipate the cells and fish claimed in the present application. The publication date of this reference is less than one year before the filing date of the U.S. Provisional Application No. 60/416,504, filed October 3, 2002, on which this application is based.

Dorsky et al. lists Richard I. Dorsky, Laird C. Sheldahl, and Randall T. Moon as authors, while only Dorsky and Moon are listed as inventors on the present application, so the disclosure of Dorsky et al. would normally qualify as prior art under 35 U.S.C. § 102(a). Dorsky et al., *Dev. Biol. 241*:229-237 (2002) (published online on February 28, 2002), is disqualified, however, as a citable prior art reference under 35 U.S.C. § 103(a). A Declaration of inventor Randall T. Moon under 37 C.F.R. §1.132 (hereinafter "the Moon Declaration") is submitted

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herewith. The Declaration is effective to remove Dorsky et al. as a citable prior art reference because the cited reference describes the inventors' own work.

Claim rejections under 35 U.S.C. § 103(a):

Claims 11-14 stand rejected under 35 U.S.C. § 103(a) as being obvious and therefore unpatentable over Dorsky et al., in view of Talbot et al., *Genes Dev. 14*:755-762 (2000).

The Examiner asserted that Dorsky et al. suggests using a transgenic fish having the limitations of the claimed zebrafish to screen to assay for mutations that affect Wnt/β-catenin signaling, even though it does not teach that the transgenic zebrafish comprise an induced mutation, induced by chemical or insertional retrovirus mutagenesis. Talbot et al., however, discusses the use of chemical and insertional retrovirus mutagenesis for the purposes of genomic analysis contemplated by Dorsky et al., with a strong statement that these two methods together "will advance the zebrafish field most rapidly in the near future." Therefore, it was asserted that it would have been obvious to one of ordinary skill in the art, and would have had a reasonable expectation of success, to use the mutagenesis methods taught by Talbot et al. to assay for mutations contemplated by Dorsky et al.

Dorsky et al. discloses the inventors' own work, as cited above.

Talbot et al. reviews recent advances that augment the cloning of mutated zebrafish genes by positional cloning, the identification of a mutated gene based on its chromosomal map position. The candidate gene approach relies on an expectation of phenotypic changes which result from disruption or other alteration which affect the expression of a known gene. Insertional mutagenesis is another approach to clone mutated zebrafish genes. Mouse retroviral vectors are used to facilitate the insertion of DNA segments into the zebrafish genome that are then identified by inverse PCR techniques. Reverse genetic approaches that rely on screens that identify mutants that fail to complement a deletion that removes a gene of interest have also been used.

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As described above, the Moon Declaration effectively removes Talbot et al. as a citable prior art reference. Talbot et al. fails to teach or suggest the claimed invention. Talbot et al., alone, is ineffective as a reference as it fails to teach, suggest, or provide a motivation to combine elements to produce the claimed invention. Accordingly, withdrawal of this ground of rejection is respectfully requested.

The Rejections of Claims Under 35 U.S.C. § 103(a):

Claim 9 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Korinek et al., *Science* 275:1784-1787 (1997), in view of Li et al., U.S. Patent Number 6,130,313.

Claim 9 as written was directed to a host cell transformed with a reporter nucleic acid comprising a DNA molecule 80% identical to a DNA molecule comprising nucleotides 2005-4336 of SEQ ID NO:1 contiguous to nucleotides 1-243 of SEQ ID NO:1. Claim 9 as written was asserted to be overly broad and indefinite, and if even if the 80% identity adjective were moved to recite the two segments of DNA, instead of the DNA molecule comprising the two segments, the amended claim would also be too broad and indefinite.

Korinek et al. was cited for teaching the construction of the pTOPFLASH vector comprising a promoter/enhancer region fused to a luciferase reporter gene, and the application teaches that the sequence 3005-4336 is comprised of the promoter/enhancer region of this plasmid fused to a nucleic acid encoding a destabilized GFP reporter gene (dGFP). Korinek et al. was also cited for teaching the use of this vector for cellular assays, including harvesting and lysis of cells for the detection of reporter gene expression, including those designed to measure β-catenin and TCF activity.

Li et al. was cited for disclosing a rapidly degrading GFP protein having improved properties for use in reporter gene assays. A portion of SEQ ID NO:2 of Li et al. is identical to a portion of SEQ ID NO:1 disclosed in the present application. An alignment was provided by the Examiner.

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The Examiner asserted that it would have been obvious to one of ordinary skill in the art, and with a reasonable expectation of success, to substitute the GFP of Li et al. for the luciferase encoded in the pTOPFLASH vector of Korinek et al.

Korinek et al. discloses the construction of a plasmid vector, designated pTOPFLASH, comprising three (3) copies of the optimal T cell factor/lymphoid enhancer factor (Tcf/Lef) motif CCTTTGATC upstream from a minimal c-*Fos* promoter driving expression of luciferase. The reporter gene assays were carried out in SW480 colon carcinoma cells. The product of the hTcf-4 gene, which is one of four known members of the Tcf/Lef family in mammals (Tcf-1, Lef-1, Tcf-3, and Tcf-4), is believed to facilitate transcription only when associated with β-catenin. This interaction is regulated by the adenomatous polyposis coli (APC) tumor suppressor protein, which binds to β-catenin. The hTcf-4 target genes have not been identified.

Li et al. discloses fusion proteins comprising an enhanced green fluorescent protein (EGFP) coupled to a peptide which produces a destabilized protein compared to EGFP alone. The "humanized" gene for EGFP is typically expressed in higher amounts in mammalian cells than the native jellyfish gene. The destabilizing peptide, called a PEST sequence, is derived from the C-terminal region of ornithine decarboxylase, a region enriched with proline, glutamic acid, serine, and threonine. The EGFP/PEST fusion proteins have a half-life in cells that is markedly decreased from that of wild-type GFP.

The instant application discloses the construction of a vector, designated TOPdGFP, suitable for use in transparent transgenic fish, such as zebrafish. A 358 bp promoter/enhancer region of pTOPFLASH, containing 4 consensus Lef binding sites and a 94 bp minimal c-*Fos* promoter, was amplified by PCR. The minimal promoter has no activity in zebrafish on its own. The 358 bp region was then inserted into the vector pCS2+, replacing the CMV promoter in this vector. A destabilized GFP, designated d2GFP, was then inserted downstream from the TOP promoter and upstream from the SV40 poly(A) site, creating TOPdGFP.

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Neither Korinek et al. nor Li et al., alone or in any combination, disclose or suggest building a vector such as TOPdGFP which comprises several genetic elements, in a specific order, which is suitable for analyzing the expression of GFP under the regulatory control of a Tcf/Lef responsive promoter in transparent, developing zebrafish. Luciferase is not suitable for some of the types of experiments contemplated by the inventors of the current application, which relies on visual examination of developing embryos, in the presence or absence of compounds that modulate the expression of genes regulated by Tcf/Lef, β-catenin, and/or other related factors. Nor do Korinek et al. or Li et al. offer any motivation to shuffle or otherwise combine various genetic elements to produce the coupled transcription unit/reporter gene disclosed in the current application. Accordingly, reconsideration and withdrawal of this ground of rejection in view of the amendments to Claim 9 are respectfully requested.

CONCLUSION

Entry of the foregoing amendments, reconsideration and withdrawal of the grounds for rejection of each of the claims, and prompt favorable action are requested. The Examiner is further requested to contact applicants' representative at the number set forth below to discuss any issues that may facilitate prosecution of the application.

Respectfully submitted,

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